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PERSPECTIVES IN BASIC SCIENCE

# Polymorphism of host response genes: Implications in the pathogenesis and treatment of acute renal failure

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**Polymorphism of host response genes: Implications in the pathogenesis and treatment of acute renal failure.** Acute inflammatory disorders are the result of an interaction between genetic and environmental factors, and are often characterized by an imbalance between pro- and anti-inflammatory host immune responses. Over the past decade, polymorphisms of host response genes have been explored as genetic risk and prognostic markers in the course and severity of acute inflammatory disorders.

Increasing evidence supports an important role for inflammatory mechanisms in the pathogenesis of acute renal failure (ARF) following both ischemic and nephrotoxic injury. The use of genetic epidemiology may become a useful tool to identify patients with an altered susceptibility to developing hospital-acquired ARF, and stratify those who may benefit from preventive therapies that modulate host immune responses in a favorable way.

This review summarizes the existing experimental and clinical studies supporting the role of inflammation in ARF and critically appraises studies that have examined polymorphism of immune response genes as potential determinants of susceptibility to and severity of acute inflammatory disorders. Conclusions are drawn on the application of genetic epidemiology to the field of ARF and the rationale for further research on the role of genetic markers in ARF.

Acute renal failure (ARF) is a serious complication that develops in hospitalized patients, with an incidence of 5% to 10% [1], and case fatality rates often exceed 50% [2]. These alarming figures have not changed in decades despite tremendous advances in supportive and dialysis care, and they have been ascribed in part to increasing age and a high prevalence of comorbid conditions. ARF has also been independently associated with increased risk of death following radiocontrast procedures [3] and following cardiac surgery [4]. Interventions to prevent or improve treatment of ARF are therefore urgently needed.

There is mounting evidence to support the critical role of inflammation in the pathogenesis of ARF. This review article is based on the premise that acute inflammatory disorders are the result of an interaction between genetic and environmental factors. Over the past decade, genetic studies of common disorders have shed some light on the relative importance of genetic risk and prognostic markers in acute and chronic inflammatory disorders.

This review summarizes the experimental and clinical data supporting the role of inflammation in ARF and critically appraises studies that have examined the importance of genetic markers as potential determinants of susceptibility to and severity of acute inflammatory disorders. Conclusions are drawn on the application of genetic epidemiology to the field of ARF.

**Key words:** acute renal failure, critical illness, infection, sepsis, inflammation, cytokines, chemokines, toll-like receptors, heat shock proteins, mortality.

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## HUMAN GENETIC VARIABILITY

Although a person's genotype represents the blending of parental genotypes, two unrelated persons share over 99.9% of their DNA sequences [5]. Many efforts both in the academic and commercial sector are under way to categorize and catalog the variations observed in the remaining 0.1% of the human genome [6]. These variants, known as gene polymorphisms, are markers of biologic diversity, and some genotypic variations correlate with specific phenotypes relevant to human disease [7, 8]. These emerging research efforts are based on the premise that human gene polymorphisms may be instrumental in developing the future practice of "genomic medicine" and individualized health care [6]. However, it is not clear whether many of these variants are incriminated in the pathogenesis of diseases, or are merely in proximity to other pathogenic genetic factors, a phenomenon known as linkage disequilibrium.

Polymorphism of human genes may be observed at one or more of the following sites (Fig. 1): (*1*) the promoter

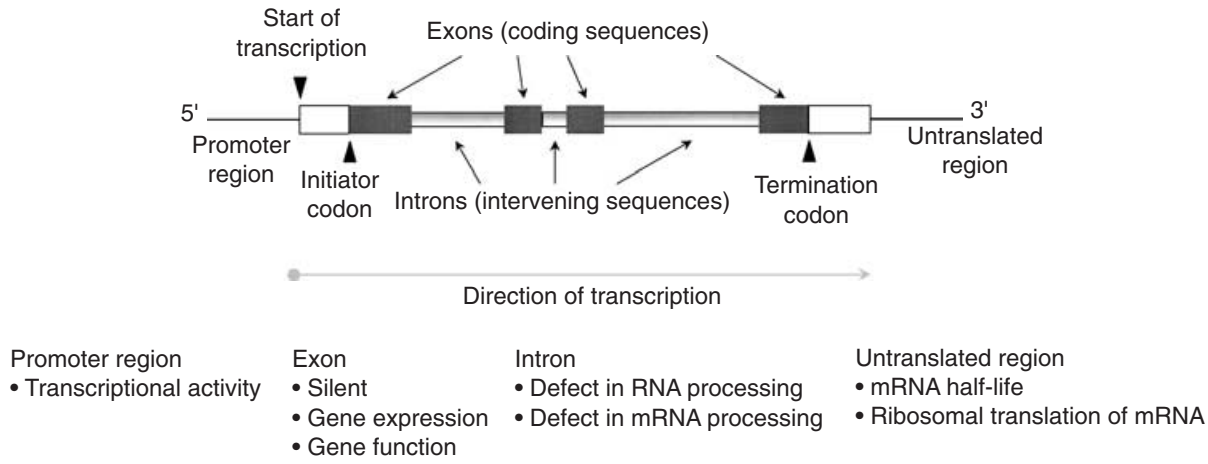


**Fig. 1. Structure of a human gene, sites of polymorphism, and functional relevance.** (Modified from [205]; reprinted with permission. Jaber BL et al: *Blood Purif* 22: 101–111, 2004).

or 5′-flanking region; (*2*) the exon(s) or the gene coding sequences; (*3*) the intron(s) or the gene intervening sequences; and (*4*) the 3′-untranslated (3′-UTR) region.

Polymorphism involving the promoter region (5′-flanking region) of the gene may affect transcriptional activity and can therefore be of functional relevance [9]. Polymorphism of the coding region or exon may be silent or affect gene expression or function by resulting in changes in the structure, binding or trafficking of the protein. Introns are transcribed into RNA but are cut out of the messenger RNA (mRNA) before it is translated into a protein molecule. Consequently, polymorphism involving the intron may lead to defects in RNA and mRNA processing. Finally, polymorphism in the 3′-UTR region may affect gene expression by affecting RNA half-life or influencing ribosomal translation of mRNA [10].

## TYPES OF HUMAN GENE POLYMORPHISM

Three types of human gene polymorphisms have been described [9] (Fig. 2): (*1*) single nucleotide polymorphism (SNP); (*2*) variable number of tandem repeats (VNTR) or minisatellite polymorphism; and (*3*) microsatellite polymorphism.

SNP is the most common class and normally consists of single nucleotide substitution (Fig. 2A). SNP in the promoter region of a gene may affect transcription factor binding, transcriptional control, or other aspects of gene expression.

Minisatellite polymorphism or VNTR results from the insertion in tandem of multiple copies of a nucleotide sequence of less than 100 bp long, known as a minisatellite, between two restriction sites (Fig. 2B). This class of polymorphism is characterized by many alleles, based on the number of minisatellite copies [9].

Microsatellites are stretches of DNA in which a short motif of one to five nucleotides is repeated several times (Fig. 2C). A dinucleotide repeat of cytosine and adenine $(CA)_n$ is the most commonly described form. Microsatellites show heritable and stable differences among individuals and are therefore polymorphic.

## IDENTIFICATION OF GENES RELEVANT TO HUMAN DISEASE

There are two approaches to identifying genes relevant to human disease: linkage analyses and association studies. Linkage analyses identify coinheritance of a specific phenotype with a region of the genome in families. These analyses are successful in identifying novel genes for "monogenic" diseases, and environmental factors tend to play a minimal role in disease expression. One such example is polycystic kidney disease. Association studies identify susceptibility genes for common "polygenic" diseases. This "candidate gene" approach relies on biologic plausibility to help identify highly likely candidate genes. In this analytical approach, gene environment interactions play a critical role in disease expression. Examples would include the study of genes encoding for cytokines in sepsis and ARF.

We will next review host immune responses in acute inflammatory disorders and specifically, the inflammatory network in ARF, with special emphasis on selected pathophysiologic pathways incriminated in sepsis-, ischemic-, and nephrotoxic-mediated injury.

## THE HOST IMMUNE RESPONSE IN ACUTE INFLAMMATORY STATES

During an acute inflammatory state, the host immune response is often governed by a balance of two opposing forces, a proinflammatory response geared toward recruitment of inflammatory cells to sites of injury and an anti-inflammatory response aimed at limiting tissue injury and promoting healing. These immune responses

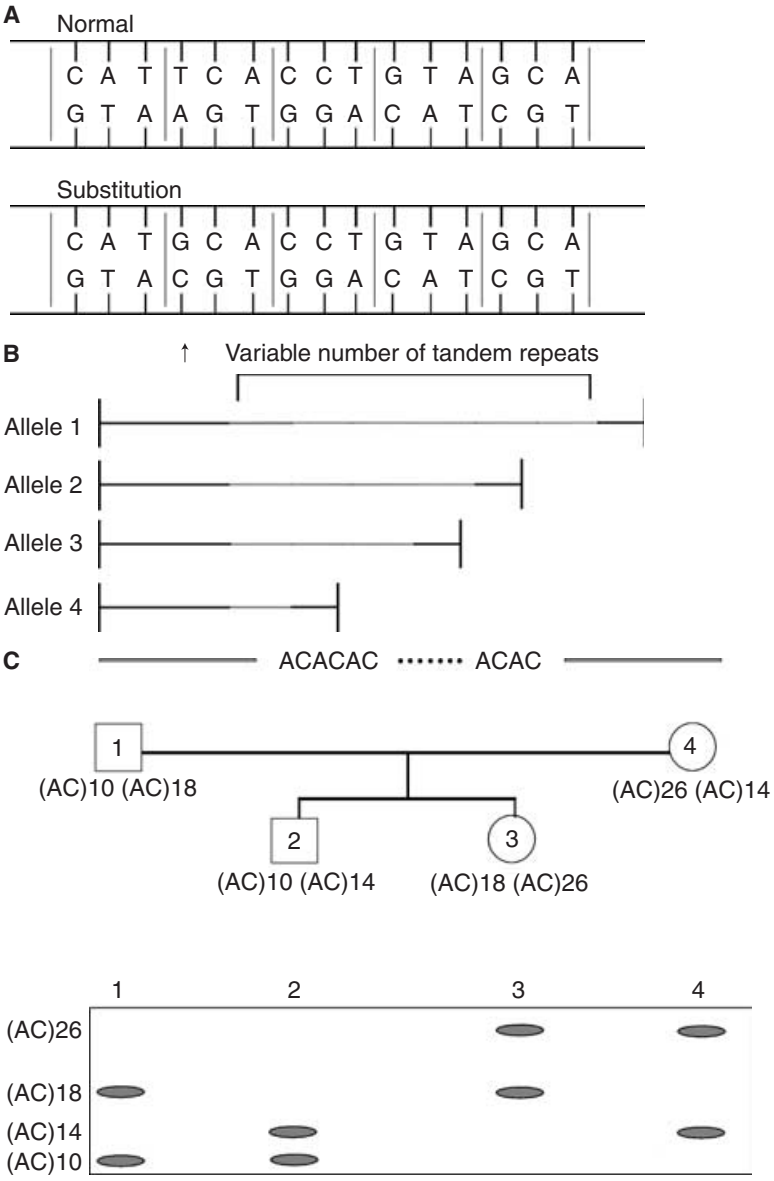


**Fig. 2. Types of gene polymorphism.** (*A*) Single nucleotide polymorphism. (*B*) Variable number of tandem repeats or minisatellite loci polymorphism (DNA sequence 10 to 100 bp in length). (*C*) Microsatellite loci polymorphism (DNA sequence of repeating units of 2 to 4 nucleotides).

are normally regulated by monocytes and lymphocytes. In bacterial sepsis, a very common acute inflammatory state, this balance has been termed the systemic inflammatory response syndrome (SIRS) and the compensatory anti-inflammatory response syndrome (CARS), and these cellular responses are usually driven by monocytes and neutrophils. The following is a summary of these emerging concepts.

## The systemic inflammatory response syndrome (SIRS)

The SIRS is an exaggerated acute host defense response to different triggering factors such as gram-negative bacterial endotoxin, trauma, and burns [11]. This leads to the release of biologically active mediators, which activate several inflammatory cascades leading to organ dysfunction, including ARF [12]. In the United States, sepsis, an example of SIRS, is increasingly identified as a common cause of morbidity and mortality, especially in the intensive care unit setting [13].

Cytokines act in concert with specific cytokine inhibitors and soluble cytokine receptors to regulate the host response. During an acute inflammatory state, stimuli cause monocytes to release such cytokines as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6), which in turn cause widespread vascular endothelial cell injury. Indeed, TNF-α and IL-1β interact with the vascular endothelium, and stimulate platelet-activating factor, prostanoids, and nitric oxide synthesis, resulting in arterial vasodilatation, hypotension, and organ dysfunction. Furthermore, interleukin-8 (IL-8), a potent chemokine, facilitates the recruitment of neutrophils to inflammatory sites, which in turn, release their toxic cellular contents, including reactive oxygen

species (ROS) and proteloytic enzymes. IL-6 also induces the hepatic synthesis of positive acute-phase proteins, such as C-reactive protein (CRP) and suppresses synthesis of negative acute-phase proteins, such as albumin [14]. TNF-α and IL-6 also contribute to a series of metabolic events leading to protein catabolism, a state of negative nitrogen balance and significant loss of lean body mass [15]. In recent years, substantial evidence has accumulated linking proinflammatory cytokines to adverse outcomes among patients with acute inflammatory states [16, 17].

### The compensatory anti-inflammatory response syndrome (CARS)

The CARS is believed to play a critical role in counterbalancing the proinflammatory responses associated with the SIRS. This secondary immune response is characterized by the production of immunomodulatory molecules that control the proinflammatory cytokine response, thereby, counterbalancing the SIRS. Their critical role in systemic inflammatory states is becoming recognized [18]. Major anti-inflammatory monocyte-derived molecules include IL-10, IL-1 receptor antagonist (IL-1Ra), and soluble TNF receptors (sTNF-R).

IL-10 is the most potent anti-inflammatory cytokine, and plays a pivotal role in the regulation of the immune response to microbial pathogens [19]. IL-10 is released by monocytes and inhibits production of TNF-α, IL-1β, and IL-6 [20]. As IL-10 expression is delayed relative to that of TNF-α, IL-1β, and IL-6, its release provides an efficient autocrine mechanism for controlling proinflammatory cytokine production by monocytes [21]. Although the precise mechanisms by which IL-10 mediates these inhibitory effects have not been determined, both transcriptional and posttranscriptional mechanisms as well as antioxidant effects have been proposed. The balance of pro- and anti-inflammatory cascades may be important determinants of the extent of host inflammatory responses and adverse clinical outcomes. Knowledge gained in the understanding of the role and regulation of IL-10 in acute inflammatory states, for instance, may lead to the development of therapeutic strategies aimed at the modulation of IL-10 synthesis. Of note, the administration of glucocorticoids has been shown to modulate the inflammatory response syndrome in favor of IL-10, resulting in a decrease in circulating TNF-α, IL-6, and IL-8 levels and an increase in IL-10 levels [22, 23].

The IL-1Ra is a member of the IL-1 family that binds to IL-1 receptors but does not transduce intracellular signals [24]. IL-1Ra is mainly secreted by monocytes in response to other cytokines and microbial components. Endogenous IL-1Ra is important in host defense against endotoxin-induced injury as well as in acute and chronic inflammatory states [25]. Studies have shown that a 1000-fold excess of IL-1Ra is required to block the hemodynamic effects of IL-1β [26]. Consequently, the balance between proinflammatory cytokines such as TNF-α and IL-1β, and their specific inhibitors such as IL-10 and IL-1Ra, respectively, may be critical in determining the extent of the inflammatory response, and may have an important influence on outcomes of patients suffering from an acute inflammatory disorder.

### Th1 and Th2 responses

Monocyte and T-lymphocyte interactions also play a critical role in the systemic response to acute inflammatory states. Two types of T-helper (Th) cells produce distinct patterns of cytokines. Th1 cells secrete interleukin-2 (IL-2) and interferon-gamma (IFN-γ), whereas Th2 cells mainly produce IL-4 and IL-10. The relative activation of Th cell subsets is determined by a variety of factors, including antigen presentation and the cytokine milieu. In sepsis, the patterns of Th1 and Th2 cell responses are not well known. In one study of healthy volunteers, after low-dose endotoxin administration, the T-cell immune response shifted toward Th2 cell type responses, with depressed circulating IL-2 levels contrasting with marked increases in IL-10 levels [27]. Although the balance of Th1/Th2 cell activation may be important in directing effector pathways and patterns of injury in acute glomerulonephritis [28], the role of lymphocytes in the pathophysiology of acute tubular necrosis is less well understood.

## THE INFLAMMATORY NETWORK IN ARF

### Sepsis injury in ARF

ARF often develops in the setting of sepsis, and the role of proinflammatory cytokines in the development of endotoxin-mediated ARF is well documented [29–31]. In sepsis, cytokine release is initiated by a number of factors, including bacterial endotoxin, ischemia/reperfusion, complement activation, and the redundant effect of other cytokines and immune modulators (Fig. 3). This results in leukocyte activation along with the expression of adhesion molecules, and the production of various biologically active substances, including oxygen-free radicals, arachidonic acid metabolites, platelet-activating factor, nitric oxide, endothelins, and heat-shock proteins (HSPs) [32, 33]. This proinflammatory cascade contributes to endothelial injury of the renal microvascular bed [34], leading to the development of tubular dysfunction and ARF. In addition, bacterial products can activate neutrophils within an already injured kidney [35], and IL-8 acts to recruit neutrophils to sites of inflammation [36]. Of note, morphologic studies of acute tubular necrosis in humans demonstrate a preponderance of neutrophils in vasa recta and the interstitium, supporting a role for inflammation in a condition that has often been thought to be noninflammatory [37].

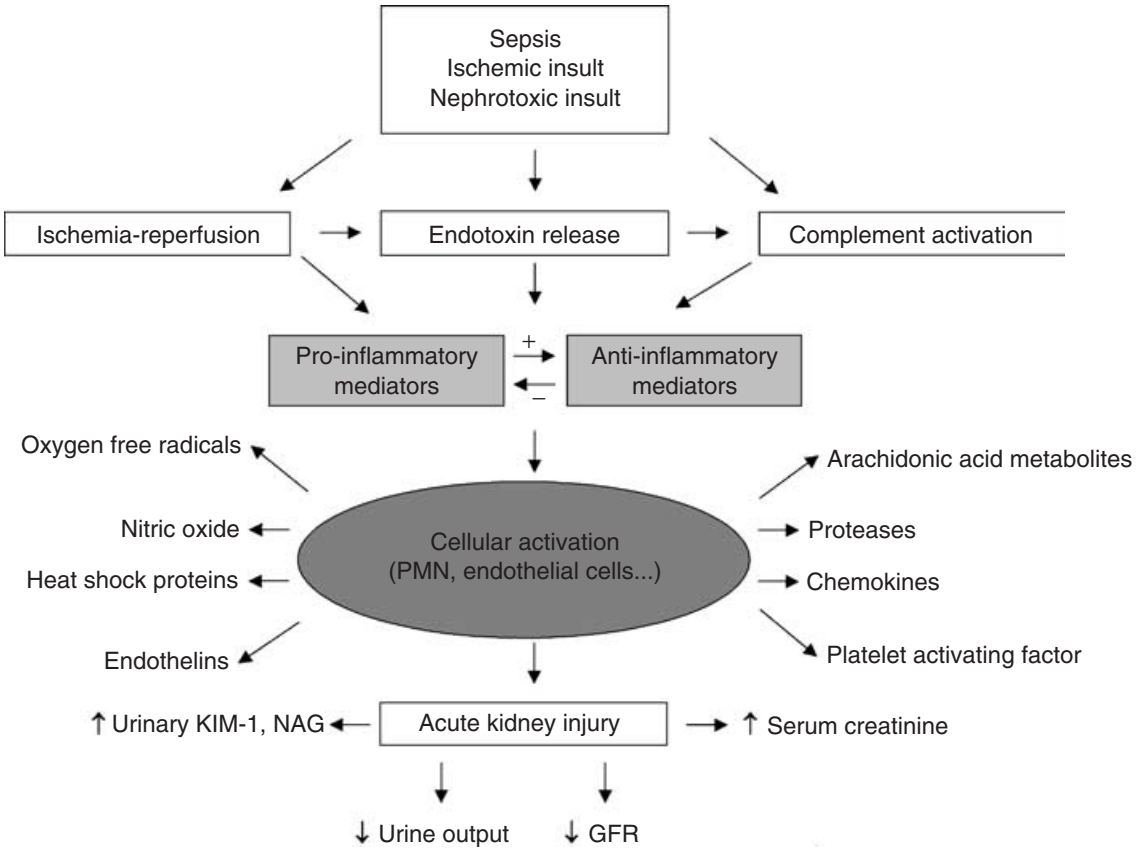


**Fig. 3. Schematic representation of the inflammatory response to sepsis and resulting kidney injury.** (Modified from [205]; reprinted with permission. Jaber BL et al: *Blood Purif* 22: 101–111, 2004). Abbreviations are: GFR, glomerular filtration rate; NAG, N-acetyl-β-D-glucosaminidase; KIM, kidney injury molecule 1.

## Ischemic and nephrotoxic injury in ARF

Although systemic or renal hemodynamic insults are important in the pathogenesis of ARF, increasing evidence supports an important role for inflammatory mechanisms in the pathogenesis of ARF following both ischemic [34] and nephrotoxic [38, 39] injury.

In a mice model of whole body ischemia, ARF is characterized by inflammation with increased renal myeloperoxidase (MPO) levels and increased gene expression of intercellular adhesion molecule-1 (ICAM-1) and IL-6 [40]. This inflammatory response as well as the development of ARF is partly reduced in T-cell–deficient mice [40], arguing for the importance of these cells in the pathogenesis of ARF following ischemia/reperfusion injury.

In a mouse model of cisplatin nephrotoxicity, ARF is characterized by the activation of proinflammatory cytokines (TNF-α and IL-1β mRNA) and chemokines [monocyte chemotactic peptide-1 (MCP-1) mRNA], and TNF-α urinary levels are increased [39]. The use of pentoxifylline, an inhibitor of TNF-α production, or a TNF-α monoclonal antibody blunts cisplatin-induced increases in TNF-α and IL-1β mRNA and reduces TNF-α urinary levels. In addition, TNF-α inhibitors ameliorate cisplatin-induced renal dysfunction and reduce structural damage. Similarly, TNF-α–deficient mice are resistant to cisplatin nephrotoxicity [39]. Overall, these data suggest that TNF-α plays a central role in these responses [39].

Chemokines, including MCP-1 and IL-8, also play a prominent role in the acute inflammatory response in several models of ARF. Indeed, following ischemia/reperfusion injury, kidney MCP-1 mRNA is increased and correlates with the extent of mononuclear infiltrate [41]. Finally, a kidney that is already primed with activated inflammatory cells may be further affected by the immunologic activation induced by the contact of blood with poorly biocompatible dialysis membranes [42].

With a growing body of evidence that there is increased expression of proinflammatory cytokines in experimental models of ARF, the role of anti-inflammatory strategies in limiting tissue injury is emerging. For instance, the exogenous administration of IL-10 inhibits TNF-α and ICAM-1 mRNA expression in mice kidneys following ischemic and nephrotoxic injury [38], and antibody to ICAM-1 protects the kidney against ischemic and nephrotoxic injury [43, 44]. The administration of IL-10 also inhibits macrophage-mediated injury in experimental glomerulonephritis by inhibiting MCP-1, IL-1β, and ICAM-1 expression [45, 46].

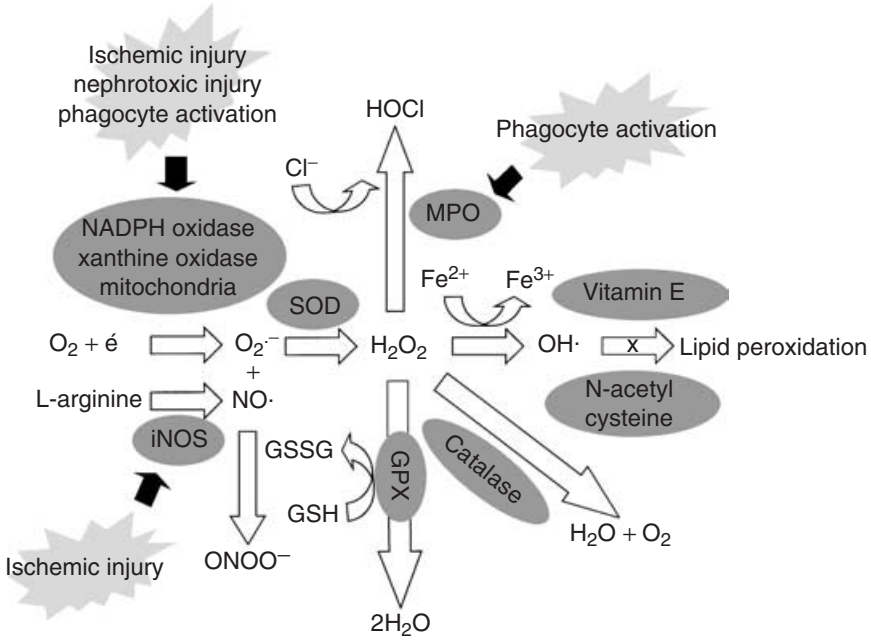


**Fig. 4. Key pathways involved in the generation and degradation of oxidants in acute renal failure.** Abbreviations are: $O_2^-$, superoxide anion; SOD, superoxide dismutase; MPO, myeloperoxidase; NO, nitric oxide; $ONOO^-$, peroxynitrite. OH·, hydroxyl radical; GPX, glutathione peroxidase; HOCl, hypochlorous acid; iNOS, inducible nitric oxide synthase.

In humans, urinary cytokine levels correlate with urinary markers of tubular dysfunction. Indeed, following surgery with cardiopulmonary bypass (CPB), changes in plasma and urinary cytokine homeostasis correlate with urinary N-acetyl-β-D-glucosaminidase (NAG) level, a biomarker of renal proximal tubular dysfunction [47, 48]. Whether urinary cytokines in humans are due to filtration by the glomerulus or production in situ is not clear. They may be involved in the pathobiology of ARF, through paracrine effects. While the role of urinary cytokines and other biomarkers of kidney injury remain unknown, a recent study demonstrated the detection of the soluble form of human kidney injury molecule-1 (KIM-1) in the urine of patients with biopsy-proven acute tubular necrosis [49]. This transmembrane protein is expressed by the human renal proximal tubule, and has been proposed as a useful biomarker for renal proximal tubule injury, to facilitate the early diagnosis of ARF [34, 50].

In humans, plasma cytokine levels have been correlated with the risk of ARF and adverse outcomes. Both plasma IL-6 and IL-10 levels have been shown to differentiate surviving from nonsurviving patients with ARF [51]. Furthermore, among patients with septic shock, elevated serum levels of sTNF-R (forms I and II) were found to be independent predictors for the development of ARF and mortality [52]. In another study of septic patients, an elevated plasma IL-6 to IL-10 ratio was associated with a higher risk of multiple organ failure, suggesting that IL-10 deficiency may contribute to organ dysfunction in sepsis [53].

## Oxidative stress in ARF

Under normal physiologic conditions, a homeostatic balance exists between the formation of ROS and their removal by endogenous antioxidant compounds [54], which reside in the intracellular space, the cellular membrane, and the extracellular space.

The reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) oxidases are membrane-associated enzymes that catalyze the production of superoxide and they are highly expressed in neutrophils and endothelial cells. [55]. NADPH oxidase has several subunits, including gp91phox and p22phox, which are electron-transfer proteins. These electron-transfer proteins are predominantly intracellular in endothelial cells, and extracellular in neutrophils [56]. This may explain cell-specific differences in the function of the two enzymes. In sepsis and possibly ARF, there are several potential sources of ROS, including the mitochondrial respiratory electron transport chain, xanthine oxidase activation as a result of ischemia/reperfusion and neutrophil-associated respiratory burst via the activation of membrane-bound NADPH oxidase [57].

MPO is released by activated neutrophils and monocytes and takes part in primary host defenses by catalyzing the production of hypochlorous acid, a potent oxidant (Fig. 4). In addition to its antimicrobial activity, MPO has been involved in a broad range of noninfectious diseases, including ischemia/reperfusion injury, vasculitis, atherosclerosis, cancer, and neurodegenerative disorders [58].

In human cells, glutathione and the glutathione peroxidases (GPX) constitute the principal intracellular antioxidant defense system [59]. There are at least four different glutathione peroxidases, all of which contain selenocysteine at their active sites [60]. Glutathione peroxidase 1 (GPX1), the ubiquitous intracellular form and key antioxidant enzyme within most cells, uses glutathione

(GSH) to reduce hydrogen peroxide to water and lipid peroxides to their respective alcohols [61], and it also reduces peroxynitrite [62]. Whereas GPX1 is particularly abundant in the kidney, GPX2 is expressed primarily in gastrointestinal tissues and GPX3 is extracellular and is present mainly in the plasma. GPX4 is detected in most tissues both in the cytosol and within membranes and has the ability to reduce peroxidized phospholipids in cellular membranes [63].

Superoxide dismutase (SOD) converts superoxide anion to hydrogen peroxide and is represented by three different ubiquitously expressed enzymes: cytosolic copper- and zinc-containing SOD (SOD1), mitochondrial manganese-containing SOD (SOD2), and extracellular SOD (SOD3). Extracellular SOD is most active in the vessel wall and has been shown to regulate the availability of nitric oxide by scavenging superoxide anion [63, 64].

Although β-carotene, lycopene, and coenzyme Q have all been implicated as cell membrane antioxidants, fat-soluble vitamin E or α-tocopherol is by far the most important free radical quencher, residing in the hydrophobic inner layer of membranes [57].

Finally, an additional line of extracellular antioxidant compounds handles the extrusion of ROS into the extracellular space that can occur, for example, following neutrophil activation. Indeed, metal-binding plasma proteins, including lactoferrin and serum albumin, via oxidizable thiol groups, which permit free radical scavenging, are valuable circulating antioxidants. Other important circulating antioxidants include vitamin C, uric acid, and bilirubin [57].

Oxidative stress occurs when a balance is disrupted by excessive production of ROS, including superoxide, hydrogen peroxide, and hydroxyl radicals, and/or by inadequate antioxidant defenses [57], including suboptimal levels of SOD, catalase, GPX, vitamins C and E, and reduced GSH (Fig. 4).

Both of these imbalances may occur in ARF. Indeed, in recent years, considerable evidence has incriminated free oxygen radicals and ROS in the pathogenesis of ischemic and nephrotoxic ARF [65]. Experimental studies indicate that deprivation of antioxidant defenses aggravates renal injury [65]. Strategies to enhance endogenous antioxidant defenses can attenuate renal injury induced by ischemic or nephrotoxic insults [65, 66]. These observations have been supported by data from clinical observational studies indicating depressed antioxidant defenses and increased oxidative stress in critically ill patients with multiple organ failure, including ARF [67–69]. Deficiencies of water-soluble vitamins and trace elements may also occur in patients with ARF undergoing renal replacement therapy [69, 70], which may further compromise antioxidant defenses. Finally, blood-membrane interactions and neutrophil activation during dialysis may lead to the intradialytic release of MPO and generation of ROS, which further exacerbate oxidative stress [69]. This may be an additional source of tissue injury as MPO activity is already enhanced in the renal parenchyma, following ischemia/reperfusion injury, reflecting on the recruitment of activated neutrophils to sites of injury [71].

The use of N-acetyl cysteine (NAC) to prevent ARF has recently emerged. This antioxidant acts primarily by scavenging oxygen free radicals, mainly hydroxyl radicals. This effect is most pronounced in the intracellular space where it also helps restore reduced glutathione stores. In humans, NAC has been shown to be beneficial in preventing contrast-induced nephropathy [72]. In experimental models of ischemic ARF, NAC ameliorates renal vasoconstriction, in part through prostaglandins and nitric oxide [73, 74].

## Nitric oxide metabolism in ARF

Nitric oxide is an important signaling molecule that is generated from L-arginine by the action of nitric oxide synthase (NOS), and has a half-life of only a few seconds in vivo. However, it is soluble in both aqueous and lipid media, and therefore, readily diffuses through the cytoplasm and plasma membranes. In the vasculature, nitric oxide stimulates the intracellular production of cyclic guanosine monophosphate (cGMP), resulting in smooth muscle relaxation and vasodilatation. In the extracellular milieu, nitric oxide reacts with oxygen and water to form nitrates and nitrites, two harmless compounds. Nitric oxide toxicity is linked to its ability to combine with superoxide anions to form peroxynitrite, an oxidizing free radical that can cause DNA fragmentation and lipid oxidation. In the mitochondria, peroxynitrite acts on the respiratory chain complex and manganese-containing SOD, to generate superoxide anions and hydrogen peroxide, respectively.

In ischemia/reperfusion injury of the kidney, nitric oxide is produced to a greater degree by tubular and interstitial cells rather than the endothelium. In addition to its role as a strong vasodilator, nitric oxide inhibits production of vasoconstrictors and reduces inflammation by neutralizing ROS [75]. Studies have found that nitric oxide derived from inducible NOS (iNOS) contributes to the preconditioning effect in the kidney [76]. Although nitric oxide is not currently used as a therapy for renal injury [77], there is evidence in humans that the use of L-arginine, the substrate for iNOS-dependent nitric oxide generation, reduces kidney injury [78].

## Summary

In summary, although animal and human studies have incriminated cytokines, chemokines, ROS, and nitric oxide in the pathophysiology ARF, the role of susceptibility genetic factors, which may influence the above mentioned

**Table 1.** Selected list of polymorphism of immune response genes in humans

| Gene | Polymorphism | | | Gene expression or function | Reference |
|---|---|---|---|---|---|
| | Site | Class | Position | | |
| Cytokines | | | | | |
| TNF-α | Promoter | SNS (G to A) | −238 | Affected | [80] |
| TNF-α | Promoter | SNS (G to A) | −308 | Affected | [82–85] |
| TNF-β (LT-α) | Intron | SNS (G to A) | +250 (intron 1) | Affected[a] | [87] |
| TNF-β (LT-α) | Intron | SNS (G to A) | +1069 (intron 1) | Affected[a] | [86] |
| IL-1α | Promoter | SNS (C to T) | −889 | Affected | [104] |
| IL-1α | Intron | 46-bpVNTR | Intron 6 | Unknown | [206] |
| IL-1β | Exon | SNS (C to T) | +3953 (exon 5) | Affected | [207] |
| IL-1β | Promoter | SNS (C to T) | −511 | Affected | [105] |
| IL-1Ra | Intron | 86-bp VNTR | Intron 2 | Affected | [111] |
| IL-6 | Promoter | SNS (G to C) | −174 | Affected | [120, 122, 124] |
| IL-6 | Promoter | SNS (G to C) | −572 | Affected | [121] |
| IL-10 | Promoter | SNS (G to A) | −1082 | Affected | [132, 133] |
| IL-10 | Promoter | SNS (C to T) | −819 | Affected | [80, 133] |
| IL-10 | Promoter | SNS (C to A) | −592 | Affected | [80] |
| Chemokines | | | | | |
| IL-8 | Promoter | SNS (A to T) | −251 | Affected | [140, 141] |
| IL-8 | 3′UTR | SNS (G to A) | +2767 | Affected | [142, 143] |
| IL-8 | Promoter | SNS (C to T) | −845 | Affected | [144] |
| MCP-1 | Promoter | SNS (G to A) | −2518 | Affected | [145, 146] |
| Toll-like receptors (TLR) | | | | | |
| TLR2 | Exon | SNS (C to T) | +2029 | Affected | [208] |
| TLR2 | Exon | SNS (G to A) | +2251 | Affected | [155] |
| TLR4 | Exon | SNS (A to G) | +896 | Affected | [152] |
| Heat shock proteins (HSP) | | | | | |
| HSP70-2 | Exon | SNS (G to A) | +1267 | Affected | [159] |
| HSP70-2 | Exon | SNS (G to A) | +1538 | Unknown | [160] |
| HSP70-Hom | Exon | SNS (C to T) | +2437 | Unknown | [160] |
| Oxidant stress-related enzymes | | | | | |
| HO-1 | Promoter | MSR $(GT)_n$ | −263, −185 | Affected | [172, 173] |
| NADPH oxidase p22phox | Exon | SNS (C to T) | +242 (exon 4) | Affected | [178] |
| NADPH oxidase p22phox | 3′UTR | SNS (A to G) | +640 | Unknown | [178] |
| MPO | Promoter | SNS (G to A) | −463 | Affected | [186, 187] |
| SOD3 | Exon | (C to G) | +637 | Affected | [188] |
| Catalase | Promoter | SNS (C to T) | −262 | Affected | [189] |
| GPX1 | Exon | MSR $(GCG)_n$ | Exon 1 | Unaffected | [190] |
| GPX1 | Exon | SNS (C to T) | +593 | Unaffected | [192] |
| GST M1 | Exon[b] | — | — | Affected | [194, 195] |
| iNOS | Promoter | SNS (C to T) | −1173 | Affected | [198] |

Abbreviations are: SNS, single nucleotide substitution; VNTR, variable number of tandem repeats; MSR, microsatellite repeats; UTR, untranslated region; HSP, heat shock protein; MPO, myeloperoxidase; SOD, superoxide dismutase; GPX, glutathione peroxidase; GST, glutathione-S transferase; iNOS, inducible nitric oxide synthase; TNF, tumor necrosis factor; IL, interleukin; HO, heme oxygenase; NADPH, nicotinamide adenine dinucleotide phosphate.

[a] TNF-α production is affected.

[b] Gene deletion prevalent in up to 50% of humans.

specific gene activation pathways, and consequently, both the development and severity of ARF following ischemic or nephrotoxic injury, has remained largely unexplored.

Polymorphisms of selected immune response genes are reviewed in the next section, with special emphasis on those that have been associated with an altered rate of gene expression. Associations between these polymorphisms and acute inflammatory states are summarized, and implications in the field of ARF are hypothesized.

## CANDIDATE GENES ALTERING THE INTENSITY OF THE INFLAMMATORY RESPONSE

The study of polymorphism of immune response genes in human disease may enhance the understanding of the cause and pathology of disease, identify potential markers of susceptibility, severity and clinical outcomes, identify potential markers for responders vs. nonresponders in therapeutic trials, identify targets for therapeutic intervention, and identify novel or improve existing strategies to prevent disease [7, 8].

Host immune responses strongly correlate with the severity and outcome of acute inflammatory and infectious states, and the extent of the inflammatory responses is in part genetically determined. In recent years, the study of polymorphism of immune response genes, including cytokines and other immune modulators, has become the subject of intense interest, as these genetic markers may be potential determinants of susceptibility to or severity of acute illnesses. Polymorphisms of key immune regulatory molecules are reviewed (Tables 1 and 2).

**Table 2.** Positive associations between polymorphism of immune response genes and acute infectious and inflammatory disorders

| Gene | Polymorphic allele | Acute illness | Reference |
|---|---|---|---|
| Cytokines | | | |
| TNF-α | −308 A-allele (TNFα2) | Sepsis/septic shock | [90–92, 97, 98] |
| TNF-α | −308 A-allele (TNFα2) | Meningococcal disease | [94] |
| TNF-α | −308 A-allele (TNFα2) | Cerebral malaria | [95] |
| TNF-α | −308 A-allele (TNFα2) | Mucocutaneous leishmaniasis | [96] |
| TNF-α | −308 A-allele (TNFα2) | Rate of body temperature normalization following cardiopulmonary bypass | [102] |
| TNF-α | −308 A-allele (TNFα2) | Neonatal acute renal failure | [130] |
| TNF-α | −308 A-allele (TNFα2) | Severe acute kidney-pancreas transplant rejection episodes | [135] |
| TNF-α | −308 A-allele (TNFα2) | Increased risk of death in dialysis-requiring acute renal failure | [103] |
| TNF-α | −238 A-allele | Malarial anemia | [209] |
| TNF-β[a] | +250 AA genotype | Septic shock in community-acquired pneumonia | [93, 163] |
| TNF-β | +250 G-allele | Prolonged mechanical ventilation following cardiopulmonary bypass | [99] |
| TNF-β | +1069 (NcO1) allele 2/2 | Septic shock following acute biliary pancreatitis | [210] |
| TNF-β | +1069 (NcO1) allele 2/2 | Higher circulating TNF-α levels following cardiopulmonary bypass | [100] |
| TNF-β | +1069 (NcO1) allele 2/2 | Increased mortality in severe sepsis | [97] |
| TNF-β | +1069 (NcO1) allele 2/2 | Susceptibility to severe posttraumatic sepsis | [98] |
| IL-1α | −889 TT genotype | Osteomyelitis | [211] |
| IL-1β | +3953 TT genotype | Osteomyelitis | [211] |
| IL-1β | −511 allele 2/2 | Febrile seizure in children | [106] |
| IL-1β | +3953 allele 2 | Increased risk of ESRD in PR3-ANCA vasculitis | [118] |
| IL-1β | −511 allele 1/2 | Survival in meningococcal disease | [212] |
| IL-1Ra | 86 bp VNTR (allele 2) | Susceptibility to sepsis | [113, 114] |
| IL-1Ra | 86 bp VNTR (allele 2) | Reduced Mantoux response to purified protein derivative of *Mycobacterium tuberculosis* | [115] |
| IL-1Ra | 86 bp VNTR (allele 2) | Increased risk of ESRD in PR3-ANCA vasculitis | [118] |
| IL-1Ra | 86 bp VNTR (allele 2) | Severity of Henoch-Schönlein purpura-associated nephritis | [116] |
| IL-1Ra | 86 bp VNTR (allele 2) | High soluble endothelial activation (von Willebrand factor and E-selectin) markers in acute coronary syndromes | [117] |
| IL-6 | −174 GG genotype | Improved survival in sepsis | [125] |
| IL-6 | −174 C-allele | Neonatal acute renal failure | [130] |
| IL-6 | −572 C-allele | Higher serum C-reactive protein level | [121] |
| IL-10 | −1082 GA genotype | Meningococcal disease | [134] |
| IL-10 | −1082 G-allele | Severity of illness in community-acquired pneumonia | [126] |
| IL-10 | −1082 G-allele | Severe acute kidney-pancreas transplant rejection episodes | [135] |
| IL-10 | −1082 GA genotype | Susceptibility to pulmonary tuberculosis | [213] |
| IL-10 | −1082 G-allele | Decreased risk of death in dialysis-requiring acute renal failure | [103] |
| IL-10 | −592 A-allele | Increased mortality in sepsis | [137] |
| IL-10 | −592 AA genotype | Decreased risk of acute GVHD and death | [214] |
| Chemokines | | | |
| IL-8 | +2767 A-allele | Henoch-Schönlein purpura-associated nephritis | [143] |
| IL-8 | −251 A-allele | Enteroaggregative *Escherichia coli* diarrhea | [140] |
| IL-8 | −251 A-allele | Respiratory syncitial virus bronchiolitis | [141] |
| IL-8 | −845 C-allele | Severity of lupus nephritis | [144] |
| MCP-1 | −2518 G-allele | Premature kidney graft failure | [147] |
| Toll-like receptors (TLR) | | | |
| TLR2 | Arg753Gln | Gram-positive bacterial sepsis | [155] |
| TLR2 | Arg677Trp | Susceptibility to lepromatous leprosy | [157] |
| TLR4 | Asp299Gly, Thr399Ile | Gram-negative bacterial sepsis | [154] |
| TLR4 | Asp299Gly | Sepsis and septic shock | [153, 156] |
| Heat shock proteins (HSP) | | | |
| HSP70-2 | +1267 GG genotype | Neonatal acute renal failure | [159] |
| HSP70-2 | +1267 AA genotype | Increased risk of septic shock | [163] |
| HSP70-Hom | +1538 CT genotype | Multiple organ failure following trauma | [160] |
| Oxidant stress-related enzymes | | | |
| HO-1 | Long $(GT)_n$ repeats | Increased susceptibility to coronary artery disease in diabetic patients | [173] |
| HO-1 | Long $(GT)_n$ repeats | Increased risk of restenosis following PTCA | [174] |
| HO-1 | Long $(GT)_n$ repeats | Increased risk for developing abdominal aortic aneurysm | [175] |
| HO-1 | Long $(GT)_n$ repeats | Increased susceptibility to pulmonary emphysema in cigarette smokers | [176] |
| NADPH oxidase p22phox | +242 CC genotype | Increased oxidized high-density lipoprotein cholesterol in type 2 diabetes mellitus | [183] |
| MPO | −463 GG-genotype | Increased prevalence of cardiovascular disease and higher circulating levels of pentosidine in ESRD | [187] |
| GST | GST M1-0 genotype | Increased risk for lung cancer in heavy smokers | [196] |
| GST | GST M1-B allele carrier | Decreased risk of delayed graft function | [197] |
| iNOS | −1173 T-allele carrier | Decreased risk of malarial complications | [198] |

Abbreviations are: ESRD, end-stage renal disease; PR3-ANCA, proteinase 3 antineutrophilic cytoplasmic antibody; PTCA, percutaneous transluminal coronary angioplasty; GVHD, graft versus host disease; GST, glutathione-S transferase; iNOS, inducible nitric oxide synthase; HO, heme oxygenase; MPO, myeloperoxidase; IL, interleukin; TNF, tumor necrosis factor; NADPH, nicotinamide adenine dinucleotide phosphate.

[a]TNF-β is lymphotoxin alpha (LT-α).

**The TNF locus**

The TNF-α and TNF-β [also known as lymphotoxin-α (LT-α)] genes are located on the short arm of chromosome 6. Polymorphism within the 5′-flanking region of the TNF-α gene at positions –238 (G to A) and –308 (G to A) have been reported, and the –308 A-allele, also referred to as the TNF-α2 allele, has been associated with high promoter activity [79–81]. Moreover, the TNF-α2 allele has been found to correlate with enhanced spontaneous and stimulated TNF-α production both in vitro [82, 83] and in vivo [84, 85].

The gene coding for TNF-β (LT-α) is located near the TNF-α gene. Single nucleotide polymorphism at position +1069 (G to A) in the first intron of the gene has been characterized [86]. The "A" variant is the TNF-β2 allele whereas the less frequent "G" variant is known as the TNF-β1 allele. The TNF-β1 allele is associated with the presence of a digestion site for the DNA sequence-specific restriction enzyme *Nco*I [87, 88]. This results in a restriction fragment length polymorphism (RFLP). Monocytes from TNF-β2 homozygous individuals produce significantly higher amount of IL-1β and TNF-α in response to stimuli [87]. A recent study has demonstrated strong linkage disequilibrium between TNF-α -308 (G to A) promoter and TNF-β *Nco*I polymorphisms, whereby heterozygosity for both TNF polymorphisms was associated with an increased TNF-α response [89]. This finding highlights the importance of linkage disequilibrium in studies examining disease association with polymorphism of individual immune response genes (see below).

Polymorphism in the TNF-α gene has been studied extensively and has been associated with adverse clinical outcomes among patients suffering from sepsis [90–92]. In one such study, the TNF-α high producer genotype (TNF-α2 allele) was strongly associated with an increased susceptibility to septic shock and mortality [90]. Furthermore, this TNF-α high producer genotype has been associated with increased morbidity and mortality from several acute infectious diseases, including community-acquired pneumonia [93], meningococcal disease [94], malaria [95], and mucocutaneous leishmaniasis [96]. Other studies have linked the TNF-β2 homozygous carriage, also associated with a TNF-α high producer genotype, to higher fatality among patients with severe sepsis [97], and increased susceptibility to severe post-traumatic sepsis [98].

A few studies have investigated the influence of polymorphisms in the TNF-α [99] and TNF-β [100] genes on the expression of TNF-α in patients undergoing coronary artery bypass graft (CABG) surgery with CPB. Some have also associated the TNF-β +250 G-allele with prolonged mechanical ventilation [99, 101] and the TNF-α –308 G-allele with a slower rate of body temperature normalization following CABG surgery [102]. In one study of patients with dialysis-requiring ARF, carriers of the TNF-α −308 A-allele had higher TNF-α production by endotoxin-stimulated whole blood leukocytes, a higher APACHE II score and a higher mortality risk [103].

It is clear that these genetically determined differences related to TNF-α gene polymorphisms may influence variations in proinflammatory cytokine responses to stressful stimuli. In patients with ARF, this may have important implications, as the extent of proinflammatory responses may dictate the severity of ARF and requirement for dialysis, as well as overall morbidity and mortality.

**The IL-1 gene family**

IL-1 is an important mediator of inflammation and tissue damage in multiple organs, both in experimental animal models of disease and in human diseases. The IL-1 gene family consists of IL-1α, IL-1β, IL-1 receptors (I and II), and IL-1Ra. The genes encoding the IL-1α, IL-1β, and IL-1Ra proteins are located on the short arm of chromosome 2, and the IL-1 receptor proteins on the long arm of chromosome 2.

An analysis of the allelic polymorphism in the promoter region of the IL-1α gene at position −889 revealed that the TT genotype significantly increased the transcriptional activity of the gene relative to the CC genotype, with a slight increase of the IL-1α mRNA and plasma protein levels [104].

A SNP has been identified within exon 5 of the IL-1β gene at position +3953 (C to T), which has been associated with regulation of gene transcription [80]. Another polymorphism has also been described within the 5′-flanking promoter region of the gene at position –511 (C to T) [105]. The IL-1β −511 polymorphism has been associated with febrile seizures in children [106], variation in CRP level [107], and severity of meningococcal disease [105].

The IL-1Ra gene contains VNTR of 86 bp within intron 2, giving rise to a penta-allelic minisatellite polymorphism. This is in linkage disequilibrium with IL-1Ra polymorphism at position +2018 within exon 2 [108]. The uncommon allele 2, which consists of two 86 bp repeats, has been associated with increased IL-1β production in vitro [109]. Some, but not all studies have also shown that this allele is associated with increased IL-1Ra production by human monocytes [110, 111]. Of note, a recent study demonstrated that IL-1β bioactivity is under the genetic control of IL-1Ra [112].

In several studies, the IL-1Ra polymorphic allele-2 has been associated with increased susceptibility to sepsis [113, 114], a reduced response to purified protein derivative of *Mycobacterium tuberculosis* (Mantoux test) [115], and with severity of nephritis in Henoch Schönlein purpura [116]. Among patients with acute coronary syndromes, the IL-1Ra allele-2 has also been associated with higher soluble endothelial activation markers (von

Willebrand factor and E-selectin) and the likelihood of a troponin-positive status compared with noncarriage [117].

Several studies have raised the question of whether polymorphism of IL-1β and IL-1Ra, known as the IL-1 gene cluster, when examined in conjunction, may have a role in the pathogenesis and/or severity of inflammatory and infectious diseases, by influencing the ratio between these two molecules. This altered ratio may lead to either an attenuated or enhanced inflammatory reaction in situ [118].

## IL-6

Interleukin-6 is a pleiotropic cytokine with both pro- and anti-inflammatory properties [119]. The IL-6 gene is located on the long arm of chromosome 7. A polymorphism has been identified within the 5′-flanking region of the IL-6 gene at position –174 (G to C) [120] and –572 (G to C) [121]. Transient transfection experiments using constructs containing either allele of the –174 5′-flanking region polymorphism indicate that the C allele results in suppression of IL-6 transcription in response to endotoxin or IL-1β [120]. The presence of the C allele has also been associated with lower serum levels of IL-6 in healthy subjects supporting the in vitro observation of reduced promoter strength [120]. In contrast, carriers of the wild-type IL-6 –174 G-allele have higher serum IL-6 levels [122].

The frequency of the IL-6 –174 C-allele has been estimated at 40% in an Anglo-Saxon Caucasian population [120]. Limited data suggest, for instance, that ethnic differences exist in regard to IL-6 genotype between Caucasian and African American populations in the United States [123].

Studies linking the IL-6 –174 promoter region polymorphism to clinical outcomes in acute inflammatory states have yielded conflicting results. In severely injured blunt trauma patients, there was no association between the IL-6 –174 promoter genotypes and ex vivo whole blood IL-6 production levels after stimulation with endotoxin [124]. In other studies, this polymorphism was not associated with increased incidence of sepsis nor did it correlate with plasma IL-6 level [125]. This polymorphism also failed to predict severity of illness in community-acquired pneumonia [126] and episodes of acute renal allograft rejection [127].

These studies contrast with a few reports demonstrating an association between this polymorphism and acute inflammatory disorders. In one study, there was a significant association between the IL-6 –174 GG homozygous genotype and improved survival in sepsis [125]. This was independent of the systemic IL-6 response, arguing for the role of other genetically linked polymorphisms that might have been the underlying cause for this association. The IL-6 –572 C-allele and −174 CC genotypes have correlated with perioperative IL-6 profiles among patients undergoing CABG surgery [128]. This contrasts with one study where among patients undergoing CABG surgery, the IL-6 –174 GG homozygous genotype was associated with higher acute phase levels of IL-6 and with longer stays in the hospital and in the intensive care unit compared with patients carrying the −174 C-allele [129].

The constellation of high TNF-α producer and low IL-6 producer genetic variants, which may lead to higher pro- and lower anti-inflammatory capacities, has been associated with an increased risk for ARF in low-birth-weight infants [130]. This is not inconceivable as high-dose IL-6 stimulates tubular regeneration in rat kidneys following nephrotoxic injury [131], thus providing an additional effect by which IL-6 may influence the course of ARF.

In summary, the variability in association between IL-6 gene polymorphism and acute inflammatory disorders may stem from its known pro- and anti-inflammatory properties and the high frequency of the wild-type in the general population (i.e., the high IL-6 production phenotype).

## IL-10

The IL-10 gene is located on the long arm of chromosome 1. Studies have suggested that 70–80% of the variation in IL-10 production is genetically determined and appears to be controlled at the transcriptional level [132]. The IL-10 5′-flanking region, which controls transcription, is polymorphic with a single base pair substitution at position −1082 (G to A) [133]. In vitro studies have identified three phenotypic secretion levels for IL-10 based on allelic substitutions (G to A) at the −1082 position: high or intermediate producers (−1082 G-allele carrier, or genotypes GG for high and GA for intermediate producers), and low producers (genotype AA) [80, 133]. These findings have been confirmed by transient transfection studies of monocytic cell lines using constructs of each haplotype [133]. Additional polymorphisms at positions –819 (C to T) and –592 (C to A) have been described but have variably been linked to regulation of gene transcription [80, 133]. Polymorphisms at these two sites are in linkage disequilibrium.

IL-10 –1082 polymorphic alleles have been associated with susceptibility to meningococcal disease and adverse clinical outcomes [134], and severity of illness in community-acquired pneumonia [126]. An increased incidence of acute kidney-pancreas transplant rejection has been observed among organ recipients, as the IL-10 production phenotype increases (low, intermediate, high), but only among those with the TNF-α low producer phenotype [135]. The role of IL-10 as a costimulatory agent for antigen-presenting cells is well known [136]. Such data support the hypothesis that the extent of the alloimmune

response following organ transplantation is in part genetically determined, and that the IL-10 high producer phenotype in this particular setting may be deleterious.

The IL-10 –592 A-allele has been associated with higher mortality in sepsis [137]. In one study of patients with dialysis-requiring ARF, the IL-10 –1082 G-allele carriage was associated with higher IL-10 production and a lower risk of death after adjustment for APACHE II score, multiple organ failure score, and sepsis [103].

### Chemokines

IL-8 and MCP-1 are two potent neutrophil and monocyte chemokines, respectively, which have been implicated in the pathogenesis of inflammation in human glomerulonephritis and acute renal allograft rejection [138]. Several studies have attempted to link interpatient variations in the extent of kidney injury to chemokine gene polymorphism, as certain polymorphic alleles may influence the level of chemokine expression in situ, thereby affecting the severity of renal injury.

The IL-8 and MCP-1 genes are located on the short arm of chromosome 4 and 17, respectively [139]. Polymorphism in the IL-8 promoter region at position –251 has been associated with both enteroaggregative *Escherichia coli* diarrhea [140] and respiratory syncitial virus bronchiolitis [141]. A polymorphism at position +2767 (G to A) in the 3′-UTR region of the IL-8 gene has been shown to contribute to its posttranscriptional regulation [142], and an increased frequency of the +2767 A-allele has been found among patients with Henoch Schönlein purpura who develop nephritis [143]. Finally, a novel polymorphism in the promoter region of the IL-8 gene at position –845, found primarily in African Americans, has been associated with the severity of lupus nephritis [144].

A genetic polymorphism in the 5′-flanking region of the MCP-1 gene at position –2518 has been shown to affect MCP-1 expression [145]. Indeed, the MCP-1 –2518 G-allele was associated with higher MCP-1 production levels by human monocytes [145]. Furthermore, the MCP-1 –2518 GG genotype has been associated with higher plasma and urinary MCP-1 levels in patients with lupus nephritis [146], and has been linked to a higher risk for premature kidney graft failure [147].

### Toll-like receptors

Toll-like receptors (TLR) are transmembrane proteins that are key mediators of the innate immune response to microbial pathogens. They are responsible for the recognition and initiation of cell-mediated immune responses, such as the release of inflammatory cytokines, phagocytosis, and the expression of costimulatory molecules needed by antigen-presenting cells. Ten different subtypes of TLRs (TLR 1 to 10) have been so far identified in mammals, and these are responsible for the recognition of separate classes of pathogens that have common specific molecular features, known as pathogen-associated molecular patterns (PAMP).

TLR2 and TLR4 have received particular attention because of their specificity for the recognition of peptidoglycans, lipoteichoic acid, and other antigens specific for gram-positive bacteria, and lipopolysaccharide (LPS) and other bacterial glycolipids specific for gram-negative bacteria [148], respectively. Genetic defects of TLR4 receptors in inbred mouse strains cause resistance to LPS challenge [149]. This lack of an inflammatory response to LPS has been reproduced with TLR4 knockout mice [150]. On the other hand, overexpression of TLR4 in transgenic mice results in an overwhelming inflammatory response after LPS exposure [151].

Polymorphisms of both the TLR2 and TLR4 gene, affecting bacterial antigen responsiveness, have recently been identified in humans (Tables 1 and 2) [152]. These polymorphic alleles have been linked to adverse clinical outcomes in sepsis [153, 154], gram-positive [155] and gram-negative [156] bacterial infections, as well as in mycobacterial infection [157].

### Heat shock proteins

HSPs are highly conserved proteins that are expressed in both physiologic and pathologic conditions. HSPs are classified on the basis of molecular weight and the 70 kD proteins referred as the HSP70 family represent the most prominent group, and include constitutive HSP73 and the stress inducible HSP72, HSP70-2, and HSP70-Hom [158–160]. Members of the HSP70 family have been incriminated in the protection against cellular damage from stressful stimuli by acting as chaperone molecules, binding to and preventing aggregation of denatured or abnormal proteins, and facilitating the restoration of normal protein function. Recent studies have shown that HSP70 proteins also act like cytokines and stimulate monocytes to produce proinflammatory cytokines, including TNF-$\alpha$, IL-1$\beta$, and IL-6 [161]. The increased expression of HSP attenuates experimental renal ischemic injury, and therefore, its modulation by pharmacologic maneuvers may be critical in inflammatory conditions and organs prior to a potential acute insult [162].

The genes encoding for HSP70 proteins are located on chromosome 6. A SNP has been identified within the exon region of the HSP70-2 gene at position +1267 (G to A) [159] and +1538 (G to A) [160]. Another polymorphism has also been described within the codon region of the HSP70-Hom gene at position +2437 (C to T) [160]. Neonates carrying the HSP70-2 (+1267) GG genotype, which is associated with low inducibility of HSP70-2, are at greater risk for developing ARF [159]. This contrasts with a study of adults with community-acquired pneumonia where the HSP70-2 (+1267) AA genotype was

associated with an increased risk of septic shock [163]. Finally, the HSP70-Hom (+1538) CT genotype has been associated with multiple organ failure following trauma [160].

### Heme oxygenase-1

Heme oxygenase (HO) is the rate-limiting enzyme in heme catabolism, which leads to the generation of biliverdin, free iron, and carbon monoxide. Three mammalian HO isoforms have been identified, one of which, HO-1, is a stress-responsive protein induced by various oxidative agents. The kidney is one of the prominent sites for intense oxidative processes in the body and is, therefore, extremely vulnerable to free radical–mediated injury [164]. Studies over the past decade indicate that HO-1 is induced as a beneficial response in cells exposed to a diverse array of toxic insults [165]. HO-1 overexpression using gene transfer protects rat livers as well as rat kidney transplants from ischemia/reperfusion injury [166]. Increased HO-1 expression is also cytoprotective in heme-mediated (e.g., rhabdomyolysis) as well as nonheme-mediated (e.g., cisplatin-induced injury) models of ARF [165]. In addition, analyses of HO-1–null mice as well as the first reported HO-1–deficient human have also emphasized the potent anti-inflammatory properties of HO-1. Both mice and humans deficient in HO-1 expression have a phenotype of an increased inflammatory state [167, 168]. Recent studies have focused on the mechanisms by which HO-1 mediates its anti-inflammatory effects. Carbon monoxide, one of the three byproducts of heme catabolism by HO, mediates potent anti-inflammatory effects through a mitogen-activated protein kinase-dependent pathway [169]. Further, other studies have shown that IL-10 induces expression of HO-1 via a p38 mitogen-activated protein kinase-dependent pathway [170]**.** The inhibition of HO-1 protein synthesis or activity significantly reverses the inhibitory effect of IL-10 on LPS-induced TNF-α production [170]. In addition, IL-10-mediated protection against LPS-induced septic shock is significantly attenuated by cotreatment with the HO inhibitor, zinc protoporphyrin [170]**.** These observations indicate that HO-1 is an important downstream effector of IL-10. The human HO-1 gene has been mapped to the long arm of chromosome 22 [171] and a highly length polymorphic $(GT)_n$ dinucleotide repeat has been identified in the proximal promoter region [172]. Transient transfection assays with HO-1 promoter/luciferase fusion constructs carrying various lengths of $(GT)_n$ repeats indicate that longer $(GT)_n$ repeats exhibit lower transcriptional activity [173]. Patients with type 2 diabetes mellitus carrying longer $(GT)_n$ repeats have been shown to exhibit higher oxidative stress and increased susceptibility to the development of coronary artery disease [173]. Long $(GT)_n$ repeats have also been associated with increased risk of restenosis after percutaneous transluminal angioplasty [174], development of abdominal aortic aneurysm [175], and increased susceptibility to pulmonary emphysema in cigarette smokers [176]. The role of this HO-1 promoter $(GT)_n$ polymorphism in modulating susceptibility to or severity of ARF has not been investigated. However, given the cytoprotective effects of HO-1 in experimental models of ARF, it could potentially be an important genetic factor influencing the pathobiology of acute renal injury.

### Oxidant stress-related enzymes

Oxygen free radicals are important mediators of cellular injury following organ ischemia and reperfusion [177]. Although polymorphisms affecting key pro- and anti-oxidant enzymes may alter the susceptibility to oxidative stress-mediated injury, the use of genetic epidemiology for the study of oxidative stress-related genes has received little attention in the setting of an acute illness [63]. We will review a selected number of polymorphisms of key oxidant stress–related genes, which may be functionally relevant in human disease.

Given its importance in generating superoxide during the respiratory burst of neutrophils, one might anticipate that polymorphisms disrupting the function of NADPH oxidase might alter oxidative stress-mediated injury in acute inflammatory disorders. Polymorphisms in the gene encoding the NADPH oxidase p22phox subunit have been described. Among them, a single nucleotide substitution (C to T) within exon 4 at position +242, resulting in an amino acid substitution at position 72 (histidine to tyrosine), has been shown to modulate enzyme activity through heme-binding affinity [178]. In one study, the p22phox +242 CC genotype was associated with higher basal and stimulated superoxide generation by human endothelial cells, compared with the p22phox +242 T allele [179]. Another single nucleotide substitution (A to G) at position +640 located in the 3′-UTR of the p22phox gene has also been described, but is of unclear clinical significance [178]. More recent studies have not supported a functional role for the A640G or C242T polymorphisms either in determining endothelial function in patients with coronary artery disease [180] or hypercholesterolemia [181]. Studies on the influence of the p22phox C242T polymorphism on circulating markers of lipid peroxidation [i.e., malondialdehyde (MDA) levels] have yielded conflicting results among patients with coronary artery disease and type 2 diabetes mellitus [182, 183].

With respect to kidney disease, the NADPH oxidase p22phox subunit is expressed by human glomerular mesangial cells, and through the generation of superoxide, may contribute to glomerular injury. Unfortunately, the p22phox C242T polymorphism has not been associated with the severity of glomerulonephritis [184]

or with the development of pre-eclampsia [185]. Despite these negative studies, the neutrophil-associated NADPH oxidase system may be extremely important in sepsis, whereby the respiratory burst during ischemia/reperfusion injury may be in part genetically predetermined. To date, no studies have attempted to link the p22phox C242T polymorphism with the susceptibility to or severity of acute organ injury in sepsis.

MPO is another neutrophil-associated enzyme, which is an important mediator of cellular injury through the generation of oxidants. Variations in the gene encoding for MPO may influence protein expression, and therefore, may be linked to pathologic states through modulation of oxidative activity. There is a well-described SNP within the promoter region of the MPO gene at position −463, consisting of a G to A substitution. The G allele (in contrast to A allele) is associated with high transcriptional activity [186]. In a recent study of patients with chronic kidney failure, carriers of the MPO –463 GG-genotype (associated with higher MPO levels) had a higher prevalence of cardiovascular disease and higher circulating levels of pentosidine, a surrogate marker of protein oxidation [187]. It remains to be seen whether polymorphism of this critical neutrophil-associated enzyme is important in dictating severity of injury following ischemia/reperfusion in ARF.

The SOD3 gene is located on the long arm of chromosome 4 and contains 3 exons. A single nucleotide substitution (C to G) within exon 3 at position +637, resulting in an amino acid substitution at position 213 (arginine to glycine), has been associated with enhanced plasma levels of extracellular SOD [188].

The catalase gene is located on the short arm of chromosome 11 and contains 13 exons. A functional polymorphism in the human catalase promoter, consisting of a C to T substitution at position –262, confers increased basal expression in different cell types and higher enzyme activity in red blood cells [189].

The GPX1 gene is located on the short arm of chromosome 3 and contains 2 exons. A microsatellite polymorphism involving a variable number of three nucleotides repeats $(GCG)_n$ (poly-alanine) in exon 1 has been described [190]. Three polymorphic alleles consisting of 5, 6, and 7 alanine repeats, however, have not been associated with altered GPX1 activity [191]. Another polymorphism, consisting of a C to T substitution at position +593, resulting in an amino acid change at position +197 (proline to leucine) has also been described, but is not associated with altered GPX1 activity [192].

Glutathione S transferases (GSTs) are soluble enzymes that play an important role in the detoxification of oxidative stress end-products including lipid and DNA hydroperoxides. There are at least four GST isoenzymes (M, P, T, and Z) with many described polymorphic alleles [63]. The gene coding for GST M1, the most studied compound, is located on the short arm of chromosome 1. Three GST M1 alleles have been identified, GST M1-0, GST M1-A, and GST M1-B [193]. Fifty percent of humans lack this enzyme (GST M1-0) [194], whereas the GST M1-A and GST M1-B alleles differ at amino acid residue 172, where lysine is replaced by asparagine, a substitution that has no impact on the enzymatic activity [195]. Smokers who are GST M1 deficient (GST M1-0) are at higher risk of developing lung cancer [196]. In one study of genetic determinants of delayed graft function following kidney transplantation, patients receiving a kidney from a donor who expressed GST M1-B either alone or in combination with GST M1-A experienced significantly lower rates of delayed graft function [197]. These promising data suggest that this antioxidant gene may be protective against ischemia/reperfusion injury following organ transplantation, and may also be applicable to ischemic and nephrotoxic ARF.

### Nitric oxide synthase

In the kidney and the vasculature, nitric oxide is produced by three major NOS isoforms: neuronal, endothelial, and inducible NOS (nNOS, eNOS and iNOS), respectively). Neuronal NOS and eNOS participate in the homeostatic control of the vascular tone, the glomerular microcirculation and other processes. In contrast, iNOS is typically induced by proinflammatory stimuli to produce large amounts of nitric oxide, which contributes to the hemodynamic collapse of sepsis and inflammatory injury responses of the glomerulus, vasculature, and the tubulointerstitium.

A single nucleotide substitution (C to T) in the promoter region of the iNOS isoform-2 (iNOS2) gene at position −1173 has been shown to affect nitric oxide expression [198]. Indeed, the iNOS –1173 T-allele carrier has been associated with increased fasting urinary and plasma nitric oxide metabolite concentrations and with a lower risk of malarial complications [198]. To date, there are no known associations between iNOS polymorphism and the development or severity of ARF.

## LIMITATIONS OF GENETIC STUDIES

Polymorphism-association studies have traditionally consisted of case-control studies, comparing the prevalence of a genetic marker in unrelated people with a given disease to the prevalence in a control healthy population. In general, such studies have been constrained by small sample sizes, with chance accounting for the results, and therefore, they must be interpreted with caution, especially when biologic plausibility has not been determined or is unknown. A better understanding of genetically linked polymorphisms may be critically important, as the polymorphism of a particular gene may

just be a marker for another, yet to be identified, disease-causing sequence variant [199]. This more comprehensive approach requires the use of linkage analyses and a good understanding of neighboring candidate genes located on the same chromosome. One such good example is the cluster of the IL-1 gene family, with several genes located in proximity on a single chromosome. Furthermore, most published studies describing the frequency of cytokine gene polymorphisms in the general population are biased due to a lack of consideration of ethnicity and geographic boundary [200]. Indeed, certain genetic variants are infrequent in some but not other ethnic groups.

The HapMap Project is an international initiative, with the long-term goal of determining the common patterns of DNA sequence variation in the human genome. This ambitious program plans to determine the genotypes of one million or more sequence variants and their frequencies in DNA samples from populations with ancestry from parts of Africa, Asia, and Europe [201]. Through these population-based genetic studies, the HapMap will allow the discovery of sequence variants that affect common disease across ethnicity and geographic boundary. This endeavor will also facilitate the development of diagnostic tools and targeted therapies.

Since genetic markers are present at birth and predate onset of disease, confounding factors play a minor role unless there is a strong interaction between these markers and an important acquired risk factor [200]. Finally, survival bias is a real concern when genetic markers are being evaluated as predictors of mortality. To circumvent this important limitation, this would require the use of case control studies with matching for age and follow-up time, or more ideally, analytic prospective cohort studies should be employed in an attempt to link polymorphic genes to clinical outcomes over time [202].

Rosenthal and Schwartz [203] have proposed four criteria that need to be fulfilled in establishing medically useful links between genetic polymorphism and human disease. These authors stressed that the genetic variant under study must cause a relevant alteration in the function or level of the gene product. The number of cases associating an allele with a particular phenotype must be large enough to be convincing. The beneficial and harmful phenotypes under study must have clear-cut clinical differences and unequivocal allelic group assignment. Finally, the plausibility of the hypothesis must be convincing.

An important criterion that has also been proposed for the study of genetic polymorphisms is the reproduction of the results in different populations. Other issues to consider when analyzing the results of SNP-association studies pertain to the population characteristics, including the choice of a control group (in case of case-control studies), the prospective or retrospective design of the study, the size, and admixture of the study population, the precise phenotypic characterization, and the effects of confounders. Finally, the demonstration of the strength of an association, the specificity of the effect, the presence of a biologic gradient and gene-dose effect and the notion of temporality all need to be considered.

**Table 3.** The future of genetic studies in acute renal failure

| |
|---|
| Large prospective cohort studies |
| Careful phenotypic characterization |
| Analysis of genetic patterns and correlation with phenotypic patterns |
| Inclusion of genetic risk into an acute renal failure-risk algorithm |
| Use in risk quantification |
| Use in targeting of therapeutic strategies |
| "GeneChip" studies |

## POTENTIAL RELEVANCE OF GENE POLYMORPHISM IN ARF

ARF is influenced by acquired risk factors such as an exposure to a nephrotoxic drug, and arguably by genetic risk factors that are present at birth. The pathobiology of ARF suggests that products of many genes act concomitantly, culminating in a beneficial or deleterious balance of pro- and anti-inflammatory molecules, which in turn, determines the extent of tissue injury. The number of recognized gene polymorphisms is growing daily. Therefore, future studies should focus on the investigation of combinations of genetic markers. Some have proposed that compilation of "genetic patterns" could be associated with different "clinical patterns" [88], such as for instance, an increased susceptibility or resistance to ARF.

The future of genetic studies in ARF is summarized in Table 3. In brief, genetic epidemiology studies may help unravel the relative importance of genetic markers in predicting the development of hospital-acquired ARF. This would require large prospective cohort studies with strong correlation between these genetic markers and the early detection of urinary biomarkers of kidney injury, a rise in serum creatinine level and dialysis requirement. In the event of successful associations between these markers and outcomes, this would provide the basis for additional studies where this genetic information could be used to stratify patients who are at the highest risk of developing ARF. More important, this knowledge may have far reaching consequences and help identify target patients in clinical trials undergoing high-risk procedures who may benefit from immunomodulatory therapeutic strategies, on the basis of their genotype profiles, with the hope of preventing or attenuating kidney injury.

## CONCLUSION

In summary, in vitro and animal studies have incriminated cytokines and other immunoregulatory molecules as important mediators of kidney injury. The relative

importance of genetic factors such as polymorphisms involving immune response genes, in influencing susceptibility to ARF and adverse clinical outcomes, has remained largely unexplored and merits full attention.

Based on the strength of associations observed in studies between gene polymorphisms and the susceptibility to or severity of acute illness and adverse outcomes, we believe that some promising and emerging candidate genes to study in ARF would include the TNF and IL-10 gene [103, 204].

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